

Reproduction in the Aggregating Sea Anemone, *Anthopleura elegantissima*

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ABSTRACT: From a sample of 240 specimens of the aggregating sea anemone, *Anthopleura elegantissima*, collected a few miles north of the Golden Gate, males and females were shown to be distributed as unisexual aggregations on the rocks.

The degree of gonadal development was measured by taking the gonad index (the ratio of volume of gonads to wet weight of anemone) every month for nearly 2 years (1959 and 1960). This showed an annual reproductive cycle, beginning in late fall or winter, and culminating in complete spawning of the population in late September.

Measurements of ovarian egg size during 1959 and 1960 corresponded well with the cycle as expressed by the gonad index.

Male and female cycles were not directly comparable on the basis of the gonad index, but identifiable males were observed over nearly the same periods as females, producing tailed sperm during the time when ovarian eggs were near their maximum size, and spawning at the same time.

THE AGGREGATING SEA ANEMONE, *Anthopleura elegantissima* (Brandt, 1835), is one of the most conspicuous and abundant intertidal animals of the Pacific coast of North America, ranging from Alaska to southern California. It is a member of the largest and most widely distributed family of anemones, the Actiniidae. Other members of the family in other parts of the world occupy very similar habitats in the same gregarious fashion. It is found firmly attached to rocks in the midtidal range from approximately 0–4.5 ft above mean lower low water level, and to higher levels where wave action is extreme (Hand, 1955). On the rocky outer coast, where it is most abundant, it occurs as single individuals or, most commonly, as aggregations of a few animals to many thousands, some of which seem interrupted only by the discontinuities of the substrate. It also occurs in bays, usually as larger, isolated individuals. The receding tide leaves these animals con-

tracted into little mounds covered with bits of rock, shells, sand grains, and other debris held by adhesive papillae on the body column.

Although abundant and conspicuous, *Anthopleura elegantissima* has only recently been critically described (Hand, 1955), and nothing has been published on its reproductive history. In fact, the only published detailed descriptions of annual gonad cycles in any coelenterates are those of Marshall and Stephenson (1933) on several reef-building corals, and Stephenson (1934) on the hydroid, *Myrionema*. For information on the structure and biology of sea anemones in general, see especially Stephenson (1928), and also Hyman (1940), and for a discussion of reproductive cycles in marine invertebrates, see the recent review by Giese (1959).

The following study of reproduction in *A. elegantissima* includes (1) evidence concerning the distribution of males and females on the rocks, and (2) the annual gonad cycle.

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MATERIALS AND METHODS

All living material used in this study was collected from an approximately 100-yd length of coastline about 4 miles by road south of Stinson Beach, Marin County, California (latitude 37° 52' N), and studies were carried out in the Department of Zoology of the University of California, Berkeley.

For the study of the gonad cycle, a population of *Anthopleura elegantiissima* was sampled at approximately 4-week intervals, except when prohibited on two occasions by inclement weather or poor tides, for a period of 21 months, from February, 1959, to October, 1960. Several individuals were taken from each of several rocks, assuring the inclusion of nearly the range of sizes to be found.

The animals were anaesthetized for several hours in a 50-50 solution of sea water and magnesium chloride isotonic with sea water. They were then injected with and preserved in Bouin's picro-formol solution.

The reproductive state was assayed by determining the ratio of the estimated volume (in milliliters) of gonadal tissue to the wet weight (in grams) of the preserved sea anemone. This ratio is referred to as the gonad index, after Bennett and Giese (1955).

Before being weighed, the preserved animals were cleaned of debris adhering to the column, cut in half longitudinally, and squeezed gently and blotted on damp paper towelling for about ½ minute to remove surplus fluid from within the coelenteron.

The gonads, of which there may be a few to more than 100 per individual, develop as thickened folds of the mesenteries between the longitudinal retractor muscles and the mesenterial filaments, and basal to the level of the pharynx. To estimate the total gonad volume, each animal was dissected to count the total number of gonads, which was multiplied by the average volume of a sample of 5 gonads. The volume of each of the 5 gonads was determined by multiplying together the length, width, and

thickness as measured by a calibrated ocular micrometer in a binocular dissecting microscope. Bits of each of the 5 sampled gonads were teased apart and squashed gently in water and examined microscopically. In all but the most immature, the gonads from each animal are identified either as ovaries by the presence of ova (18 to about 200 microns in greatest diameter) with prominent nuclei, or as testes by the presence of vesicles filled with small round spermatocytes (3 to 4 microns), or with tailed sperm. Even in the field maturing females can be distinguished by the presence of the light chocolate-brown ovaries when the animal is torn open. However, even nearly mature males (with tailed sperm in the testes) must be preserved and dissected to distinguish the milky-white testes from the tangled masses of mesenterial filaments.

To further characterize the reproductive state of the females, the greatest diameter of the largest ovum found in each of the squashed bits of ovary was measured by means of a calibrated ocular micrometer in a compound microscope and the average was recorded.

For the study of the distribution of males and females on the rocks, a sample of 240 individuals was taken on July 1, 1961. From each of 12 rocks 2 samples of 10 animals each were taken from closely packed aggregations on extreme sides of the rock, noting the approximate distance between the 2 samples of 10, and whether they were part of the same or of separate aggregations. In the laboratory the animals were relaxed, preserved, and subsequently weighed and dissected; and the sex of those with gonads was determined.

DISTRIBUTION OF SEXES

While processing the animals collected in 1959 for the study of the gonad cycle, it was observed that all but a few individuals with gonads were unquestionably females, giving a sex ratio of approximately 7.5 females to 1 male. So on October 5, 1960, 100 animals were collected from the population, 2 groups of 10 from each of 5 rocks, as a larger sample for determining the sex ratio in the population. However the population had apparently entirely spawned out, as no individuals were found to bear gonads (2 weeks earlier, many animals sampled had

TABLE 1
DISTRIBUTION OF MALES AND FEMALES IN 24 GROUPS OF 10 ANIMALS
EACH COLLECTED ON 1 JULY 1961

ROCK NO.	SAMPLE	FREQUENCY			MEAN WT. OF SAMPLE IN GRAMS	DISTANCE A TO B IN FEET	CONTINUOUS OR DISCONTINUOUS
		♂	♀	NO GONADS			
1	a	—	10	—	1.09	7	Disc.
	b	4	—	6	0.73		
2	a	—	10	—	1.33	3	Disc.
	b	—	10	—	0.91		
3	a	—	6	4	0.92	2.5	Disc.
	b	1	—	9	0.80		
4	a	—	10	—	0.77	3	Disc.
	b	—	7	3	0.72		
5	a	—	—	10	0.44	12	Disc.
	b	—	3	7	1.53		
6	a	—	10	—	1.18	4	Cont.
	b	—	10	—	0.61		
7	a	—	5	5	3.11	6	Disc.
	b	—	1	9	0.47		
8	a	—	5	5	2.10	4	Disc.
	b	—	7	3	2.06		
9	a	—	4	6	0.91	2	Cont.
	b	—	4	6	1.25		
10	a	—	—	10	0.89	1.3	Disc.
	b	9	—	1	3.19		
11	a	7	—	3	2.37	4	Disc.
	b	4	—	6	0.77		
12	a	8	—	2	2.89	12	Cont.
	b	5	—	5	2.81		
Total of Individuals		38	102	100			
Per cent of Individuals		15.8	42.5	41.7			
Number of Samples of 10		7	15	2			
Per cent of Samples		29.2	62.5	8.3			

very large gonads), thus delaying this study until the following summer. When the animals collected in 1960 for the study of the gonad cycle were all dissected, the observed sex ratio was 1.8 females to 1 male. Thus it was suggested not only that the sex ratio might be other than 1:1, but that males and females might not be distributed at random within the aggregations, or in the population as a whole.

The data from the collection of the 240 animals taken on July 1, 1961, shown in Table 1, suggest the reason for the observed sex ratios. The rocks, numbered 1 to 12, were selected in a generally north to south sequence over the area from which animals had previously been collected for the study of the gonad cycle, thus including many of the same aggregations previously sampled.

The most important fact realized from the data in Table 1 is that in no case were both an identifiable male and an identifiable female found in the same sample of 10 animals. In fact, in 25% of the samples, every individual was identified as a female. Assuming a random distribution of males and females, the probability of a single sample of 10 animals being all of one sex is $\frac{1}{2}$ to the 10th power, and of 5 samples of 10 being all of one sex is $\frac{1}{2}$ to the 50th power. Thus it is apparent that the sexes are not randomly distributed, but aggregated into separate or perhaps contiguous groups of males and females.

What is not evident from the data presented is the size of the unisexual aggregations, as they were not sampled randomly but in patches about 8 to 12 cm across. What is needed to estimate the size of the unisexual aggregations and even to show whether all continuous masses of anemones are of but one sex, is a series of random samples of say, 10 animals each, from a number of continuous aggregations. It should be noted that the 3 pairs of samples taken from continuous masses (on rocks 6, 9, and 12) yielded but one sex in each case, and that in the 2 cases in which both sexes were found on the same rock (Nos. 1 and 3), the pairs of samples were taken from separate aggregations.

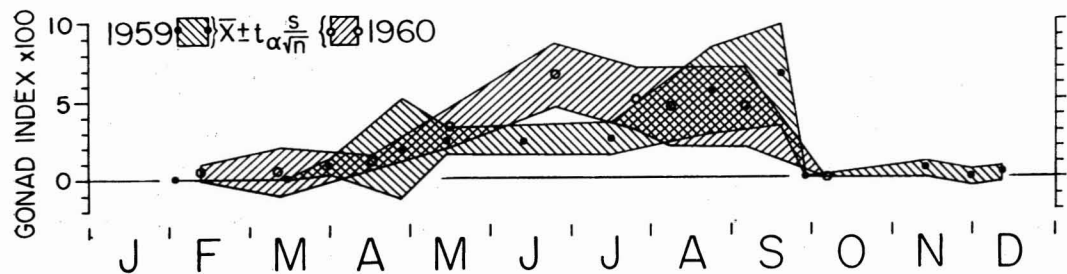
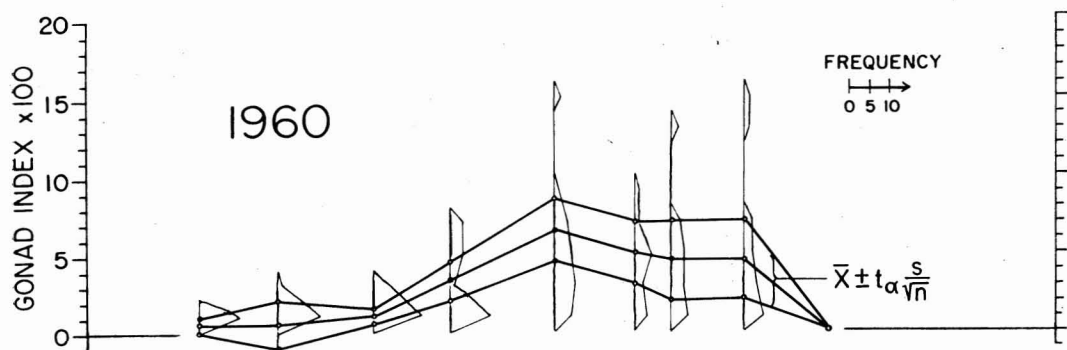
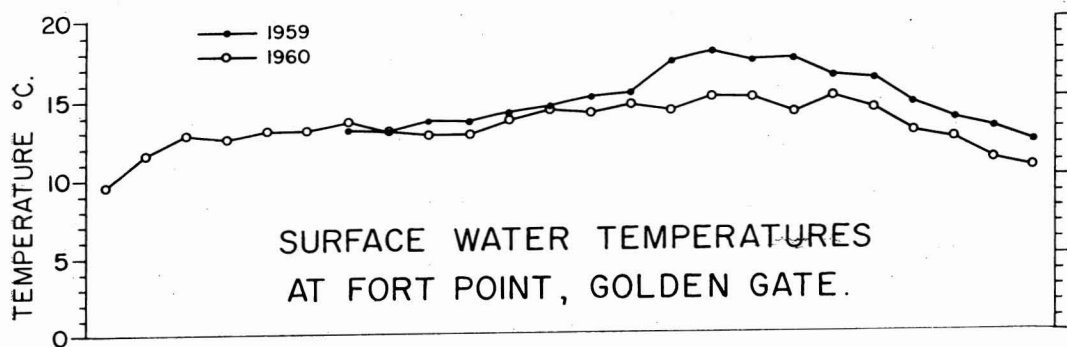
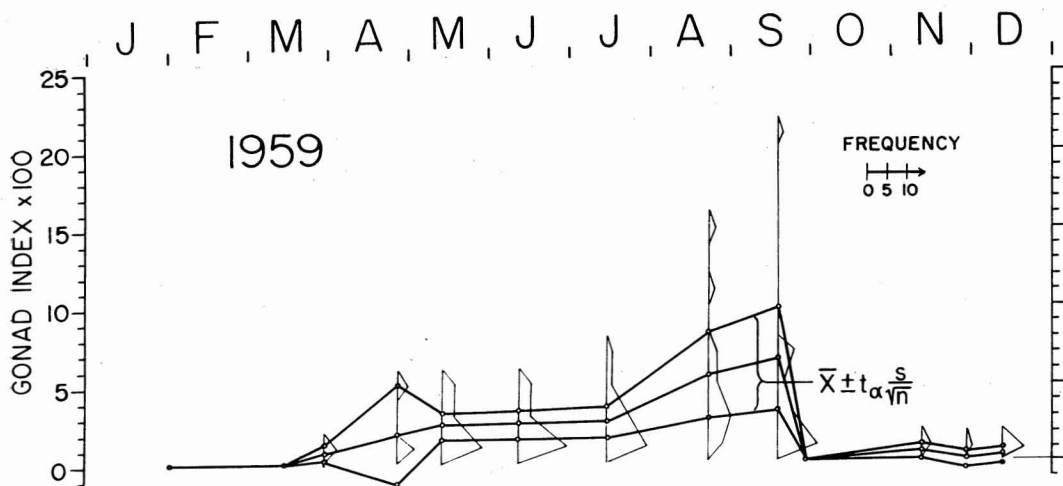
To estimate the sex ratio, there are two ways to manipulate the data in Table 1. If the data are considered to comprise a sample of 240 individuals, the sex ratio is 102 identifiable as females to 38 males, or about 2.7:1, which for this sample size (140), deviates very significantly from a 1:1 ratio. However, if each group of 10 animals is considered to be a part of a separate asexually produced clone, and is treated as but a single individual, the sex ratio of identifiable animals is 15 females to 7 males, which for this sample size (22) does not deviate significantly from a 1:1 ratio, and suggests a need for sampling a larger number of aggregations, perhaps at other locations.

Thus the clumped distribution of the sexes, and the apparent predominance of females in the population, explain in part the sex ratios observed in samples of animals collected in 1959 and 1960 for the study of the gonad cycle. The marked increase in the frequency of males observed in 1960 over 1959 (indicated in Fig. 3)

might well be explained by the unfortunate fact that the same rocks were not sampled in both years. Until mid-July, 1959, animals were taken from rocks in the area of rocks 1 through 6 of Table 1, which are in the northern half of the collecting area and adjacent to a small sandy beach to the north. In July, 1959, the sand level rose to cover most of the rocks from which animals had been collected. It should be noted that samples from rocks 1 through 6 consisted of mostly females and but a few males. Animals taken during the remainder of 1959 were taken in the area of rocks 5 through 9, samples from which yielded only females. The sand level did not rise to cover the anemones in the southern half of the collecting area, so in 1960 animals were collected from the area of rocks 6 through 12, from which a much larger proportion of males were taken. Thus the unfortunate circumstances of sampling from a population in which the sexes are not randomly distributed are offered as the most probable explanation for the dearth of males in 1959 collections, and their sporadic occurrence in 1960.

It is interesting to speculate about the significance of the clumped distribution of the sexes with regard to the origin of the aggregations in the population. It could be argued that, as is suggested above, unisexual aggregations are actually asexually produced clones, arising by binary fission from a single individual. In fact, throughout the year a few scattered individuals are observed to be pulling apart in the process of fission, and there are usually a few individuals in each sample collected showing a light-colored vertical scar on the column indicating a recent division. Hand (1955) and others (personal communication) have reported seeing populations of *A. elegantissima* in which almost every anemone is dividing or has just divided, during the months of January to March.

One might also suggest that the animals are in unisexual aggregations as the result of an active process of movement of individuals. However, when a solidly packed aggregation is removed from the substrate except for a small patch of individuals in the center, and the patch is observed over a period of weeks, the tendency is for the animals to quickly spread out, separating most of the individuals. Also, animals placed closely together in tanks in the laboratory sepa-



rate from one another and cease to wander further within a few days. This would tend to suggest that individuals are aggregated into large masses only as a result of growth and asexual reproduction within the limitations of space.

A third explanation might suggest some factor influencing larval settling, such as some substance released by larvae which first settled, which attracted larvae of the same sex and repelled those of the opposite sex. However, considering the relatively great distances involved, and the large volumes of moving sea water through which these substances would have to pass, this seems to be the most improbable explanation.

ANNUAL GONAD CYCLE OF *A. elegantissima*

The data for the gonad index of the aggregating sea anemone for 1959 and 1960 are given in Figure 1. It is evident that only a single breeding cycle occurred during each year, with gonads small during the winter months, and increasing during spring and summer to a peak before spawning out completely in September. Overlap of 95% confidence bands about the sample means indicates a generally good correlation between the cycles of the 2 years, except that the maximum gonad index may have been reached 2 months earlier in 1960 than in 1959.

The maximum ovum diameters for each sample for the two years are presented in Figure 2, which shows an almost continuous increase in ovarian egg size, from very small during the winter months to a maximum size just before spawning in September.

In both 1959 and 1960, tailed sperm were observed in July in smears of the testicular tissue of one or two males, and by mid-August all testes were filled with tailed sperm.

There seems to be some slight correlation between size and sexual reproduction, as shown in Table 1 and observed throughout the year,

in that a greater proportion of the smaller individuals (weighing 0.3 to 3.0 g) are found to be without gonads than are larger individuals (3 to 10 g). However even the smallest animals may be fertile and the largest animals may be without gonads.

If any correlation may be safely drawn between the events of the gonad cycle and ocean temperatures, as shown in Figure 1, it is that gonad size and ovarian egg size increase with the rising temperatures of the summer months, and spawning occurs shortly after temperatures have reached a peak in early fall and begun to decrease. It must be noted, however, that the temperature data were taken at the Golden Gate, several miles south of the collecting area. Most importantly, due to their position in the intertidal zone, these animals spend nearly half the time out of water, exposed to much greater temperature extremes and fluctuations than are indicated in Figure 1.

The data for male and female reproductive cycles for 1959 and 1960 are given in Figure 3. However, no attempt is made here to compare male and female cycles on the basis of these data, because of the circumstances of sampling as explained above under the topic of the distribution of the sexes.

DISCUSSION

The present account reports a single gonad cycle per year for the aggregating sea anemone, *Anthopleura elegantissima*, with the gonad index and ovarian egg size increasing gradually from a low in the fall after spawning, to a high the following September, when spawning again occurs. Spawning was not observed by the author, nor are there any published accounts of spawning in this species, but reports of such observations have been received by personal communication. These reports place the time of spawning near the end of summer, which agrees with the present findings, and state that the majority of individuals in the population were shedding

FIG. 1. Gonad cycle of *Anthopleura elegantissima* in 1959 and 1960, and corresponding ocean temperatures. Temperature data were obtained from the U. S. Dept. of Commerce, Coast and Geodetic Survey, Bureau of Marine Data. Each sample is plotted in a frequency histogram, with heavy lines joining the means and 95% confidence limits computed for a "t" distribution for small samples (Wilks, 1948). Below, the 95% confidence bands for the two years are compared.

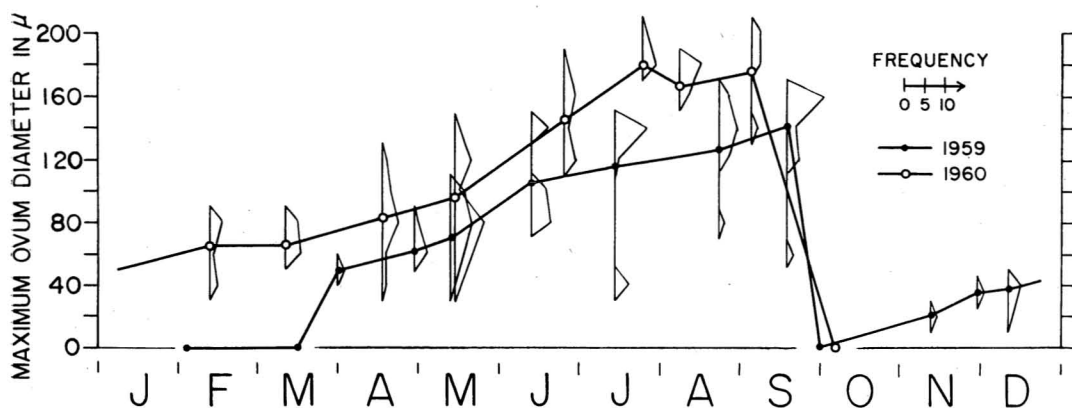


FIG. 2. Average maximum ovum diameters measured in 1959 and 1960. Samples of 25 animals each, taken on February 3 and March 3, 1959, and October 5, 1960, and a sample of 100 individuals taken on September 30, 1959, had no gonads and hence no ova.

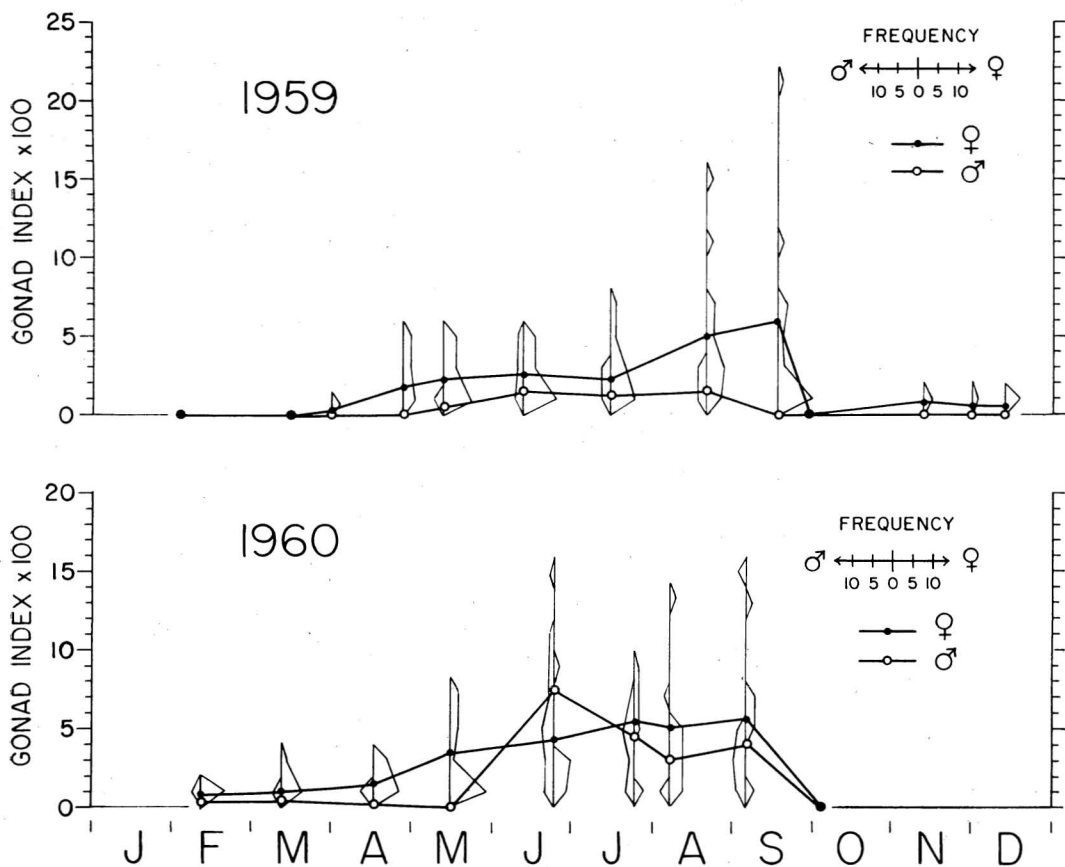


FIG. 3. Male and female gonad cycles of *A. elegantissima* for 1959 and 1960 (not strictly comparable due to circumstances of sampling).

either brownish mucous masses of eggs or milky-white masses of sperm.

There were no indications of hermaphroditism. Neither were there any suggestions that this species broods its young internally, as reported by Atoda (1954) for a Japanese *Anthopleura*.

Further work needs to be done to determine the origin of the aggregations of anemones on the rocks, and to determine the relative importance of sexual and asexual reproduction in maintaining a population in a given location. The distribution of the sexes into groups of males and females should prove to be a useful tool and point of reference for such study.

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